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Antibacterial Activity of Propolis Produced by *Trigona* spp. Against *Campylobacter* spp.

AMIN FATONI[1], I MADE ARTIKA[1]*, AHMAD ENDANG ZAINAL HASAN[1], KUSWANDI[2]

[1]Department of Biochemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Darmaga Campus, Bogor 16680, Indonesia
[2]Animal Husbandry Research Institute, Jalan Pajajaran, Bogor 16151, Indonesia

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Propolis is believed to have antimicrobial, anti-inflammatory and immunostimulating activities. The objective of this study was to investigate the antibacterial activity of ethanol extract propolis (EEP) of *Trigona* spp. from Bukittinggi West Sumatera against *Campylobacter* spp. Antibacterial activity of the EEP was measured by disc diffusion method. The compound groups of the propolis were also analyzed on the existence of alkaloids, flavonoids, saphonins, tannins, steroids, and terpenoids. This study revealed that the EEP of *Trigona* spp. shows an antibacterial activity on *Campylobacter* spp. The compound groups detected in the EEP were flavonoids and tannins, suggesting that the antibacterial activity of propolis of *Trigona* spp. may be due to these compounds.

Key words: *Trigona* spp., antibacterial activity, *Campylobacter* spp.

INTRODUCTION

Propolis (bee glue) is a sticky dark-colored material that honeybees collect from living plants, mix with wax and use in construction and adaptation of their nests (Bankova *et al.* 2000). Bees use propolis not only as a building material, but also as a means of maintaining low levels of bacterial and fungal concentrations in the hive. The action against microorganisms is an essential characteristic of propolis and it has been used by human beings since ancient times for its pharmaceutical properties. Propolis possesses antibacterial, antifungal, antiviral properties, and many other beneficial biological activities: anti-inflammatory, antiulcer, local anesthetic, hepatic-protective, antitumor, and immune-stimulating. For this reason, propolis is widely used as a popular remedy in folk medicine, in apitherapy, as a constituent of "biocosmetics", "health food", and for numerous further purposes (Bankova *et al*. 2000).

Generally propolis is obtained from honeybee *Apis* spp. One of the other bees collecting less honey and more propolis is *Trigona* spp., a member of stingless bees. Hasan (2006) found that propolis from *Trigona* spp. was effective against *Bacillus subtilis*, *Staphilococcus aureus*, and *Eschericia coli*. Sabir (2005) showed that flavonoids *Trigona* sp. propolis inhibited *Streptococcus mutans* growth.

Campylobacter is a type of pathogenic bacteria found in the intestines of many wild and domestic animals. The bacteria are passed in their feces, which can lead to infection to humans via contaminated food, meats, water taken from contaminated sources (streams or rivers near where animals graze), and milk products that have not been pasteurized. Once inside the human digestive system, *Campylobacter* infects and attacks the lining of both the small and large intestines. Infection with a *Campylobacter* species is one of the most common causes of human bacterial gastroenteritis (Moore *et al.* 2005).

Thermo-tolerant *Campylobacter*, especially *Campylobacter jejuni*, belongs to the most frequent etiological agents of food-borne diseases, the number of which has been increasing recently worldwide (Allos 2001).

The disorder usually recedes without antimicrobial therapy, however in more serious cases treatment is necessary (McDermott *et al*. 2005). The drug of choice is a macrolide (e.g., erythromycin) for the treatment of enteric campylobacter infections after microbiological diagnosis. However, for the empiric treatment of adults with suspected bacterial gastroenteritis, the drug of choice typically includes a fluoroquinolone (e.g., ciprofloxacin) because of their activity against almost all enteric bacterial pathogens (Allos 2001; Engberg *et al.* 2004).

However, it has been shown that in the course of previous years there have been selected strains of *Campylobacter* spp. resistant to antimicrobial agents, especially to fluoroquinolones (Thakur & Gebreyes 2005; Larkin *et al*. 2006). Antimicrobial drug resistance in *Campylobacter* infections, in particular to quinolones, has increased dramatically in many countries (Engberg *et al*. 2001).

Based on the reasons mentioned above, it is considered to be important to search for new antibacterial agents that can effectively inhibit *Campylobacter* growth. Little data are currently reported on the activity of propolis against *Campylobacter*. We suggest that propolis from *Trigona* spp. may be one of new natural antimicrobial agents which can be used to treat *Campylobacter* infection. Therefore, the main objective of this research was to identify antibacterial activity of *Trigona* spp. propolis against *Campylobacter* spp.

*Corresponding author. Phone/Fax: +62-251-8423267, E-mail: imart171@yahoo.com

MATERIALS AND METHODS

Sample Collections. *Trigona* spp. hive as propolis resource was collected from Bukittinggi West Sumatera during the dry season (July-August). This location resides in 100.210-100.250 °LE and 00.760-00.190 °PS, altitude 909-941 m above sea water, temperature 16.10-24.90 °C. This location has rainfall at 2.381 mm per year and humidity between 82.0-90.8% (PEMKOT Bukittinggi 2007).

Extraction of Propolis. The hive was cut into small pieces, grounded and extracted with 70% ethanol (1:5 w/v) in a shaker (EYELA, Japan) at speed of 130 rpm, and at room temperature for 14 days. The extract solution was then filtered through a filter paper, and then the ethanol was evaporated using freeze-drier to obtain ethanol extract of propolis (EEP) free of solvent (Hasan 2006).

Preparation of Inoculums. *Campylobacter* was cultured for 24 h at 42 °C in a liquid medium (campylobacter base, DIFCO) and used as inoculums. The turbidity of the suspension was adjusted to 0.5 with McFarland turbidity standard.

Antibacterial Activity. Antibacterial activity of EEP was investigated by the disc diffusion method (Andrews 2001). The bacterial screening was performed by using *Campylobacter* agar base (DIFCO) and *Campylobacter* supplement. Sterile paper discs (Whatman # 4 paper, 6 mm diameter) were loaded with 15 ìl of propolis extract dilutions (16.7, 8.3, 4.1, 2.0, 1.0, 0.2, 0.1, and 0.06% w/w). Six discs were put on each petridish cultured with *Campylobacter*. The ampicillin (100 ug/ml) and commercial propolis were used as positive controls, and the solvent was used as a negative control. Plates were incubated at 42 °C for 48 h in an anaerobic jar flowed with CO_2 and N_2. Inhibitory zone diameter was measured with a calliper each treathment was performed in triplicates. The data were subjected to analysis of variance using general liner model procedure of SPSS with α 0.05.

Compound Groups Test. The EEP was subjected to phytochemical analysis for the presence of compound groups such as flavonoids, alkaloids, terpenoids, steroids, saphonins, and tannins, based on Harborne (1996). Flavonoids were identified by using solution containing magnesium, amyl alcohol and concentrate hydrochloride acid. Alkaloids were identified by using Dragendorff solution, whereas steroids and terpenoids were tested by using Lieberman-Buchard solution. The presence of tannins was identified by using ferrichloride solution, and saphonins were identified by using foam forming test in hot water.

RESULTS

The EEP obtained was very sticky with dark brown colored. The average of extraction yield was 24.66% (w/w). The EEP still contained ethanol in very low concentration (0.05%). The diameters of bacterial growth inhibited by different concentration of EEP and controls were shown in Figure 1. The results showed that at a concentration of 16.67%, EEP was more effective than the commercial propolis, but less active compared to ampicillin (100 ppm) on *Campylobacter* spp. growth (Figure 2). The solvent (negative control) did not

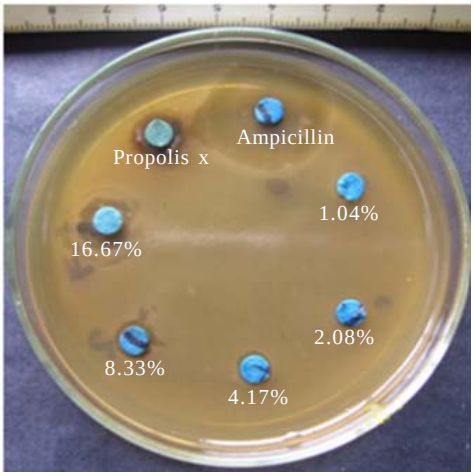

Figure 2. EEP tested on plate cultured with *Campylobacter.* 1.04%, 16.67% are EEP in concentration of 1.04% (w/w), 16.67% (w/w). Ampicillin means ampicillin solution in water at concentration 100 ppm. Propolis x is commercial propolis undilution.

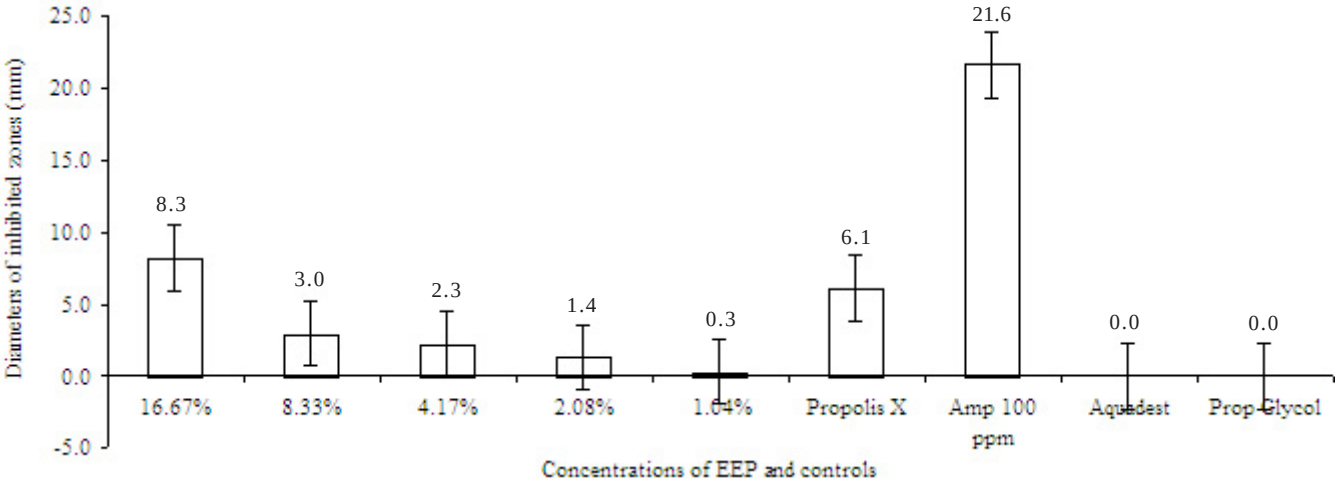

Figure 1. The means of diameters (mm) of bacterial growth inhibited by different concentrations of *Trigona* spp. propolis and other controls.

show any inhibitory effect on the tested bacteria. Among the series concentration of EEP tested, the least concentration that still showed inhibitory effect was 2.08% (two replicates), and 1.04 (one sample), thus the average of minimum inhibition concentration (MIC) was 1.73% (w/w). The compounds groups identified in *Trigona* spp. propolis are flavonoids and tannins (Table 1).

Table 1. Compound groups test results for *Trigona* spp. propolis

Compound	Test results	Color produced by test
Flavonoids	+++	Yellow
Alkaloids	-	
Saphonins	-	
Tannins	+++	Greenish violet
Steroids	-	
Terpenoids	-	

+: identified by visual observation (+++: strong, ++: fair, +: slightly); -: unidentified by visual observation.

DISCUSSION

The propolis activity and chemical compositions depend on plant species, season of propolis harvesting and geographical location of bee hive collected (Bankova *et al.* 2000; Banskota *et al.* 2000), thus researchers use these reasons to investigate propolis active compounds. The pharmacological activities of propolis are more numerous in tropical regions than that in temperate climates, due to the richer vegetal diversity observed in the former (Bankova 2005). There is few information about activity of propolis from Indonesia, especially of *Trigona* spp. bee. Sabir (2005) investigated the activity of flavonoids from *Trigona* spp. propolis collected from South Sulawesi on *S. mutans.* Hasan (2006) stated that *Trigona* spp. propolis activity from Pandeglang-Banten against *S. aureus, B. subtilis,* and *E. coli.* However, the yield obtained was less (8.2%: 8.2 g EEP per 100 g hive) than yield of this research (24.66%). The difference may be due to the diversity of plants around the *Trigona* spp. hive and the time of hive collection.

The antibacterial activity of EEP of *Trigona* spp. at 16.67% (w/w) was stronger than that of commercial propolis which may be due to two reasons. Firstly, this propolis has higher antibacterial activity than the commercial propolis. Secondly, this propolis maybe more concentrated than the commercial propolis (unknown active compound concentration). The antibacterial activity of EEP of *Trigona* spp. at 16.67% (w/w) was weaker than that of ampicillin (100 ppm), indicating that the propolis has low antibacterial activity, or *Campylobacter* spp. is more susceptible to ampicillin than to *Trigona* spp. EEP. These results agreed to many researchers that showed antibacterial activity of propolis is lower than that of ampicillin (Katircioglu & Mercan 2006; Gonsales *et al.* 2006). Although *Trigona* spp. EEP has relatively low activity on *Campylobacter*, it can be suggested as a new effective antibacterial agent for *Campylobacter* for two reasons. Firstly, it has been shown that strains of *Campylobacter* spp. were resistant to other antimicrobial agents, especially to fluoroquinolones (Thakur & Gebreyes 2005; Larkin *et al.* 2006), whereas the complexity and synergistic effects of compounds in propolis make bacteria difficult to build tolerant for propolis (Mizrahi & Lensky 1997). Secondly, propolis is a relatively non toxic drug (Nikulin *et al.* 1979).

The compound analysis showed the propolis of *Trigona* spp. was rich in polyphenol compounds, i.e. flavonoids and tannins. These results confirm previous studies reporting that caffeic acids, flavonoids and phenolic esters were the main biologically active compounds in propolis (Kujumgiev *et al*. 1993; Park *et al*. 1998; Marcucci *et al*. 2001; Kartal *et al*. 2003). However, their biological effects cannot be attributed solely to these components since the chemical composition of propolis is complex. Some authors attributed the complex composition of propolis as a reason for its antimicrobial activity, and some mechanisms of action have been proposed (Simuth *et al.* 1986; Strehl *et al.* 1994; Takaishi & Schilcher 1994; Mirzoeva *et al.* 1997; Park *et al.* 1998). The antimicrobial properties of propolis related to the synergistic effect of its compounds (Santos *et al*. 2002). The propolis affects the cytoplasmic membrane and inhibits bacterial motility as well as enzyme activity (Mirzoeva *et al*. 1997). Propolis exhibits bacteriostatic activity against different bacterial genera and can be bactericidal in a high concentration (Mirzoeva *et al*. 1997; Drago *et al*. 2000). The mechanism of action of antibacterial properties of flavonoid is by interfering bacterial cell wall permeability, microsome, and lysosome as a result of its interaction with bacterial DNA (Wilson & Gisvold 1982; Bryan 1982). This mechanism of action differs from that of ampicillin as the standard antibiotic. Ampicillin inhibits bacterial cell wall synthesis by binding to one or more of the penicillin binding proteins (PBPs); which in turn inhibits the final transpeptidation step of peptidoglycan synthesis in bacterial cell walls, thus inhibiting cell wall biosynthesis. Bacteria eventually lyse due to ongoing activity of cell wall autolytic enzymes (autolysins and murein hydrolases) while cell wall assembly is arrested (Donowitz & Mandell 1988).

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REFERENCES

Allos BM. 2001. *Campylobacter jejuni* infections: update on emerging issues and trends. *Clinical Infect Dis* 32:1201-1206.

Andrews JM. 2001. Determination of minimum inhibitory concentrations. *J Antimic Chemoth* 48 Sppl S1:5-16.

Bankova V. 2005. Chemical diversity of propolis and the problem of standardization. *J Ethnopharmacol* 100:114-117.

Bankova VS, Castro SL, Marcucci MC. 2000. Propolis: recent advances in chemistry and plant origin. *Apidologie* 31:3-15.

Banskota AH, Tezuca K, Midorikawa K, Matsushige K, Kadota S. 2000. Two novel cytotoxic benzofuran derivatives from Brazilian propolis. *J Nat Prod* 63:1277-1279.

Bryan LE. 1982. *Bacterial resistance and suspectibility to chemotherapentic agents*. Sydney: McGraw-Hill Co. p 20-24.

Donowitz GR, Mandell GL. 1988. Beta-lactam antibiotics. *N Engl J Med* 318:419-426.

Drago L *et al*. 2000. In vitro antimicrobial activity of propolis dry extract. *J Chemother* 12:390-395.

Engberg J, Aarestrup FM, Taylor DE, Gerner-Smidt P, Nachamkin I. 2001. Quinolone and macrolide resistance in *Campylobacter jejuni* and *E. coli*: Resistance mechanisms and trends in human isolates. *Emerg Infect Dis* 7:24–34.

Engberg J, Neimann J, Nielsen EM, Aarestrup FM, Fussing V. 2004. Quinolone-resistant *Campylobacter* infections in Denmark: Risk factors and clinical consequences. *Emer Infect Dis* 10:1056-1063.

Gonsales GZ, Orsi RO, Fernandes Jr A, Rodrigues P, Funari SRC. 2006. Antibacterial activity of propolis collected in different regions of Brazil. *J Venom Anim Toxins incl Trop Dis* 12:276-284.

Harborne JB. 1996. *Metode Fitokimia*. Edisi ke-2, cetakan ke-2. In: Padmawinata K, Soediro I (ed). Bandung: ITB. Translate from: *Phytochemical Methods.*

Hasan AEZ. 2006. *Potensi Propolis Lebah Madu Trigona* spp. *sebagai Bahan Antibakteri*. Seminar Nasional HKI, Bogor.

Kartal M, Yıldız S, Kaya S, Kurucu S, Topçu G. 2003. Antimicrobial activity of propolis samples from two different regions of Anatolia. *J Ethnopharmacol* 86:69-73.

Katircioglu H, Mercan N. 2006. Antimicrobial activity and chemical compositions of Turkish propolis from different regions. *African J Biotechno* 5:1151-1153.

Kujumgiev A, Bankova V, Ignatova A, Popov S. 1993. Antibacterial activity of propolis, some of its components and their analogs. *Pharmazie* 48:785-786.

Larkin C *et al*. 2006. Antibiotic resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from hog, beef, and chicken carcass samples from provincially inspected abattoirs in Ontario. *J Food Protect* 69:22-26.

Marcucci MC *et al.* 2001. Phenolic compounds from Brazilian propolis with pharmacological activities. *J Ethnopharmacol* 74:105-112.

McDermott PF, Bodeis-Jones SM, Fritsche TR, Jones RN, Walker RD. 2005. Broth microdilution susceptibility testing of *Campylobacter jejuni* and the determination of quality control ranges for fourteen antimicrobial agents. *J Clin Microbiol* 43:6136-6138.

Mirzoeva OK, Grishanin RN, Colder PC. 1997. Antimicrobial action of propolis and some of its components: the effect on growth, membrane potential and motility of bacteria. *Microbiol Res* 152:239-246.

Mizrahi A, Lensky Y. 1997. *Bee Products: Properties, Appication and Apitherapy*. London: Plenum.

Moore JE *et al*. 2005. *Campylobacter*. *Vet Res* 36:351-382.

Nikulin IM, Lisitsyna LY, Tiknonov AI, Shcherbina VD, Stebliuk PN. 1979. Propolis in the treatment of Inflammatory diseases of the airways. *Zh Ushn Nos Gorl Bolezn* 4:9-12.

Park YK *et al*. 1998. Antimicrobial activity of propolis on oral microorganisms. *Curr Microbiol* 36:24-28.

[PEMKOT] Pemerintah Kota Bukittinggi Sumatera Barat. 2007. *Peta wilayah* dan *Kondisi geografis*. http://www.bukittinggikota.go.id/null [17 Mar 2008].

Sabir A. 2005. Aktivitas antibakteri flavonoid propolis *Trigona* sp. terhadap bakteri *Streptococcus mutans* (*in vitro*). *Dent J* 38:135-141.

Santos FA *et al.* 2002. Antibacterial activity of Brazilian propolis and fractions against oral anaerobic bacteria. *J Ethnopharmacol* 80:1-7.

Simuth J, Trnovsky J, Jelokova J. 1986. Inhibition of bacterial DNA-dependent RNA polymerases and restriction endonuclease by UV-absorbing components from propolis. *Pharmazie* 41:131-132.

Strehl E, Volpert R, Elstner EF. 1994. Biochemical activities of propolis-extracts. III. Inhibition of dihydrofolate reductase. *Z Naturforsch Sect C Biosci* 49:39-43.

Takaisi, Schilcher H. 1994. Electron microscopic and microcalorimetric investigations of the possible mechanism of the antibacterial action of a defined propolis provenance. *Planta Med* 60:222-227.

Thakur S, Gebreyes WA. 2005. Prevalence and antimicrobial resistance of *Campylobacter* in antimicrobial-free and conventional pig production systems. *J Food Protect* 68:2402-2410.

Wilson, Gisvold. 1982. *Kimia farmasi dan medisinal organik*. 8th Ed. Fatah AM (ed). Jakarta: Dirjen Dikti dan Kebudayaan 10-12.